**CYTOLOGY**

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H.I.R. Chapter 3

This laboratory is divided into light and electron microscopic portions with some significant overlapping. You are expected to integrate all the information about cellular structures at both levels. Remember, you are also responsible for all demonstration materials on the bulletin boards and academic intranet site.

The electron microscopic portion consists of a series of demonstration micrographs **POSTED ON BULLETIN BOARDS IN THE BOOKSTORE HALLWAY**. Look for the various cellular components, which are indicated. Do not attempt to memorize each micrograph (there will be too many!). Instead, look for the common characteristic structures of the organelles and realize how this may vary from cell type to cell type. For example, one can easily recognize a mitochondrion by its cristae enclosed by an outer membrane. The demonstration micrographs show how mitochondria vary in shape, size, and number from one cell type to another, while the main characteristic structure still remains.

Students should study the virtual slides listed below to recognize the indicated critical cytological features. Use the histology atlas and demonstrations to help you correctly identify the diagnostic features of the various cells, tissues and organs.

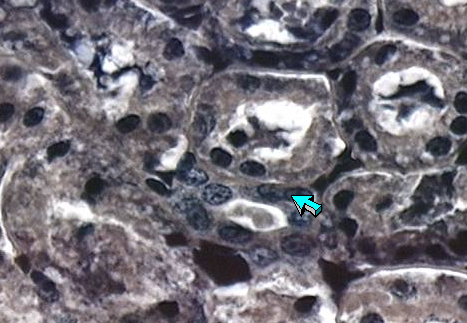
**YOU ARE RESPONSIBLE FOR ALL DEMONSTRATION MATERIALS THROUGHOUT THE YEAR** .

Do not attempt to understand the material as a tissue today. **LOOK AT CYTOLOGICAL FEATURES ONLY. Today we deal with cells just as cells.**

Begin viewing a slide images at LOW POWER . At low power, quickly scan the entire section. Later, when you begin to acquire more knowledge regarding organs, this procedure will allow you to direct your attention immediately to the area of greatest interest. Scan the entire field in an orderly fashion from top-to-bottom, and left-to-right, noting the overall cell organization. Proceed to use higher power views as necessary.

**MITOCHONDRIA**

[**[MCO 0004]**](https://www.utdl.edu/WebSlides/launch.php?name=_BLIvf2_MCO0004&type=ax&section=)Mitochondria [**Java**](https://www.utdl.edu/WebSlides/launch.php?name=_BLIvf2_MCO0004&type=jv&section=)

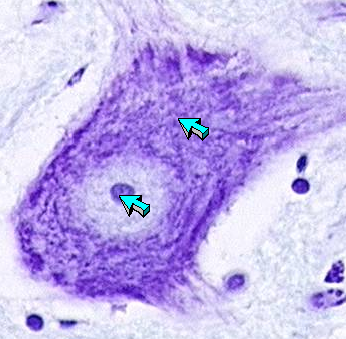


In this view of the kidney, the mitochondria are specifically stained using Regaud's method (iron alum). Find a good cross-section of a tubule in the darker staining periphery of the tissue (bottom of section). Once you have located the appropriate cells, you will have to use **high power** for resolution of the mitochondrial clumps. They appear as black dots or rods in the **basal aspect** (attached side) of these tubule cells. Many of the cells are too highly stained, and the mitochondria appear just as large dark areas, rather than individual organelles. It may take the concentrated examination of several tubule cells to find some good examples.

**RIBOSOMES AND ROUGH ENDOPLASMIC RETICULUM**

[**[MCO 0035**](https://www.utdl.edu/WebSlides/launch.php?name=_BLIvf2_MCO0035&type=ax&section=)**]** Nissl bodies and substance [**Java**](https://www.utdl.edu/WebSlides/launch.php?name=_BLIvf2_MCO0035&type=jv&section=) HIR *-frame 505*

Nucleolus and Rough ER



Locate the large multipolar neurons near the central region of the tissue using low power. These neurons will appear as large cells (blue staining masses) with varying numbers of cell processes (axons and dendrites) which extend only a short distance from the cell bodies. When observed at high power, the Nissl bodies (Rough ER and Ribosomes) appear as deep blue staining granules throughout the cytoplasm, except in the region of the axon and axon hillock. The nucleolus is stained so darkly because it is the site of r-RNA synthesis.

**GOLGI APPARATUS**

[**[MCO 0005**](https://www.utdl.edu/WebSlides/launch.php?name=_BLIvf2_MCO0005&type=ax&section=)**]** Golgi Apparatus [**Java**](https://www.utdl.edu/WebSlides/launch.php?name=_BLIvf2_MCO0005&type=jv&section=)

[**[UTN 005**](https://www.utdl.edu/WebSlides/launch.php?name=_BLIvf5_UTN005&type=ax&section=)**]** Golgi Apparatus (silver) [**Java**](https://www.utdl.edu/WebSlides/launch.php?name=_BLIvf5_UTN005&type=jv&section=)



This preparation of the dorsal root ganglion has been impregnated with a silver solution to demonstrate the Golgi apparatus in neurons. At low magnification, the darker staining round structures near the periphery of the tissue (and bottom of slide in **UTN 005**) are nerve cells (neurons). **With high power**, observe the small black spots in the cytoplasm of the neurons which represent the Golgi apparatus. These are usually in the region very near the nucleus. The nucleus is unstained and can be visualized as a round clear area surrounded by black flecks (Golgi) in the cytoplasm. Note the **crescent shaped** Golgi structure. This organization is much more clearly observed in the EM demonstration micrographs.

**MICRO FILAMENTS**

[**[LH 0046**](https://www.utdl.edu/WebSlides/launch.php?name=_BLIvf1_LH0046&type=ax&section=)**]** Skeletal muscle [**Java**](https://www.utdl.edu/WebSlides/launch.php?name=_BLIvf1_LH0046&type=jv&section=) HIR*-frame 18*



[**[UTN 079**](https://www.utdl.edu/WebSlides/launch.php?name=_BLIvf5_UTN079&type=ax&section=)**]** Tongue Cornification, Cat (H&E) [**Java**](https://www.utdl.edu/WebSlides/launch.php?name=_BLIvf5_UTN079&type=jv&section=)

 (very faint striations)

This is a cross section of tongue, which is primarily composed of muscle tissue. Find an area where the muscle cells are cut longitudinally. Observe the cross striations under high dry power. These striations are due to the registration of the thick and thin filaments in the muscle cells. These filaments are arranged in parallel arrays along the length of the cells. They are in bundles called myofibrils within these muscle cells. Compare these MICROFILAMENTS with those seen at the EM level. Details of their arrangement will be studied in much greater detail in the lecture and laboratory on Muscle Tissue.

**GLYCOGEN AS A CELL INCLUSION**

**[**[**MCO 0002]**](https://www.utdl.edu/WebSlides/launch.php?name=_BLIvf5_UTN079&type=ax&section=) Liver, Glycogen [**Java**](https://www.utdl.edu/WebSlides/launch.php?name=_BLIvf2_MCO0002&type=jv&section=) HIR - *frame 120*



This slide utilizes a glycogen stain plus a counterstain. As a result, glycogen is observed as red, round spheres within the cells. It may be difficult to see cell borders in this section, but it should be easy to see the small, round clumps of glycogen.

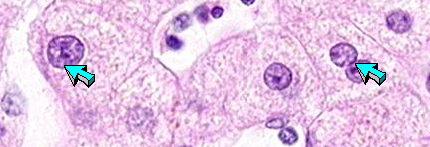
**NUCLEAR STRUCTURE AND FUNCTION**

In this section of the laboratory, we now concentrate on the structure of the cell nucleus. As you look at the various initial slides, pay particular attention to the overall size of the nucleus relative to the general cell size, as well as its location within the cell. Observe the chromatin patterns in the nuclei, euchromatin, heterochromatin, etc., as well as the prominence of the nucleolus. Also note differences in general nuclear structure, such as the "cigar shaped" nuclei of smooth muscle cells, or the segmented nuclei of white blood cells. Later in the microanatomy component of Block II, these characteristics will become very important in helping you identify specific cell types.

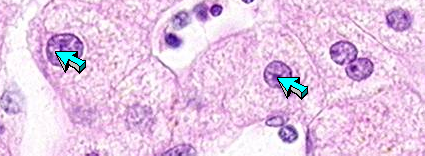
The last slide in today's exercise contains four sections of fish blastula undergoing active cell division. Take some time with this slide to identify examples of each of the 4 stages of mitosis, as well as cytokinesis. In many cases, the sections are serial sections of a single blastula. Therefore, if you locate a particular structure in one section, you may be able to follow it in 3-D in adjacent sections. If you are able to do this, you will gain an appreciation of the 3-dimensional aspects of these cells.

**INTERPHASE NUCLEI AND NUCLEOLI**

[**[MCW 084]**](https://www.utdl.edu/WebSlides/launch.php?name=_BLIvf3_MCW084&type=ax&section=)Liver, H&E [**Java**](https://www.utdl.edu/WebSlides/launch.php?name=_BLIvf3_MCW084&type=jv&section=) HIR -*frame 1026*



(Binucleaded cell and heterochromatin region of nucleus)

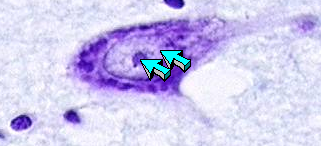


(Nucleolus and Euchromatin region of nucleus)

[**[UW 061]**](https://www.utdl.edu/WebSlides/launch.php?name=_BLIvf7_UW061&type=ax&section=) Liver, Human [**Java**](https://www.utdl.edu/WebSlides/launch.php?name=_BLIvf7_UW061&type=jv&section=)

Find the round, basophilic (blue) stained nucleus in the cells. Identify the small dark round structure or structures within the nucleus. Liver cell nuclei are characterized by a vesicular chromatin pattern. Pick out regions of heterochromatin (clumped) and euchromatin (dispersed) in the karyoplasm. It is possible that binucleated cells will be present.

[**[MCO 0035]**](https://www.utdl.edu/WebSlides/launch.php?name=_BLIvf2_MCO0035&type=ax&section=)Nissl Bodies, Neurocytes [**Java**](https://www.utdl.edu/WebSlides/launch.php?name=_BLIvf2_MCO0035&type=jv&section=) HIR *-frame 15, 43*

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(Nucleus and Nucleolus)

Return to the large multipolar neurons near the central region of this tissue. The nucleus is the clear, round area in the center of the cell. The nucleolus is the round, dark staining body in that clear area.

**CHROMATIN AND NUCLEAR STRUCTURE**

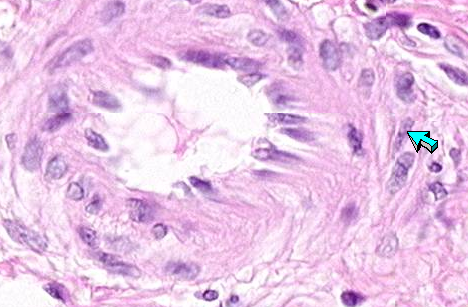
[**[MCW 084]**](https://www.utdl.edu/WebSlides/launch.php?name=_BLIvf3_MCW084&type=ax&section=)Liver, H&E [**Java**](https://www.utdl.edu/WebSlides/launch.php?name=_BLIvf3_MCW084&type=jv&section=) HIR -*frame 1026*

[**[UW 061]**](https://www.utdl.edu/WebSlides/launch.php?name=_BLIvf7_UW061&type=ax&section=) Liver, Human [**Java**](https://www.utdl.edu/WebSlides/launch.php?name=_BLIvf7_UW061&type=jv&section=)

[**[UTN 002]**](https://www.utdl.edu/WebSlides/launch.php?name=_BLIvf5_UTN002&type=ax&section=)Mammal Liver Kupffer's (H&E) [**Java**](https://www.utdl.edu/WebSlides/launch.php?name=_BLIvf5_UTN002&type=jv&section=)



(Erythrocytes)



(Different shaped nuclei around blood vessels in the liver)

In contrast to the other liver slide (**MCO 0002**), this one is probably stained by H & E. Therefore, the cytoplasm (not glycogen) is stained red. The nuclei of liver cells are the pale blue staining spheres located in the center of the red cytoplasm. Look for different shaped nuclei in the area just around a blood vessel. Try to find flattened, elongated, spiral or spindle shaped nuclei. Be able to characterize their chromatin patterns. Look within the lumen of a large blood vessel. The red- to orange- stained cells are enucleated red blood cells.

[**[MCO 0058]**](https://www.utdl.edu/WebSlides/launch.php?name=_BLIvf2_MCO0058&type=ax&section=)Artery, Vein & Nerve, Elastic Tissue, C S [**Java**](https://www.utdl.edu/WebSlides/launch.php?name=_BLIvf2_MCO0058&type=jv&section=) HIR-*frame 593*

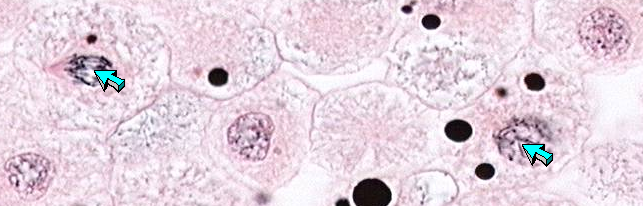
[**[MCO 0658]**](https://www.utdl.edu/WebSlides/launch.php?name=_BLIvf2_MCO0658&type=ax&section=) Artery and Vein, Elastic Tissue [**Java**](https://www.utdl.edu/WebSlides/launch.php?name=_BLIvf2_MCO0658&type=jv&section=)

 (contracted smooth muscle in artery with twisted nucleus)

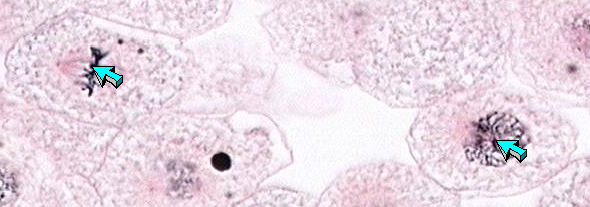
Find a medium sized artery on this slide; it will have an open lumen. Look in the middle region of the arterial wall for smooth muscle cells. There should be 8 or more layers of smooth muscle cells in the arterial wall. The twisted structure of these cell nuclei of cells in the contracted state are evident at high power.

**MITOSIS, CHROMOSOMES, ASTERS, NUCLEI, SPINDLES, AND CENTRIOLES**

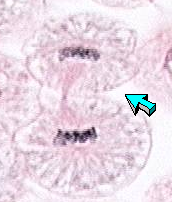
[**[MCO 0001]**](https://www.utdl.edu/WebSlides/launch.php?name=_BLIvf2_MCO0001&type=ax&section=) Animal Mitosis, Fish Blastula [**Java**](https://www.utdl.edu/WebSlides/launch.php?name=_BLIvf2_MCO0001&type=jv&section=) HIR -*frame 133 – 139*



(Prophase and early anaphase)



(Prophase and Metaphase)



(Late Telophase)



(Early Telophase)



(Late Anaphase)

Locate and characterize the nucleus and the four stages of mitosis.

The Interphase nucleus is vesicular. A nuclear membrane is still visible. Remember the important preliminary stages in the Cell Cycle. Remember what occurs during the G1, S, and G2 phases.

Prophase is marked by the condensation of chromatin into discrete chromosomes. These appear as fine squiggly threads. Know what other events occur.

During Metaphase, chromosomes are lined up in the center of the spindle at the equatorial plate. Remember how the chromosomes are attached to the spindle fibers. Know when the chromosome attaches to the spindle fibers. Know the composition of the spindle fibers. This phase is also the best opportunity to observe some classical mitotic spindles.

Anaphase is marked by the migration of the chromosomes toward the opposite poles of the spindle. You should be able to see good examples of both the early and late stages of anaphase.

Telophase is marked by the formation of the cleavage furrow. Look for early and late stages. In some sections, you should be able to identify a midbody. Know what structures form the midbody.

Define cytokinesis. Know what is occurring to the nucleus at this time. Know whether cytokinesis is a parallel or serial event.

Centrioles are not usually observed at the L.M. (light microscopic) level. However, if one observes a cell in metaphase, anaphase, or early telophase, the mitotic poles (where the centrioles are located as confirmed by TEM) are easily observed. The best chance to see this is in metaphase, where the mitotic spindle is sometimes very clearly seen. In these small cells, note the spindle fibers, which are microtubules extending from the poles.